의학박사 학위논문

# 기관지천식의 발생과 악화에 있어서 Interleukin32의 역할

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2017년 8월

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이 논문을 권 재 우 박사학위논문으로 제출함

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# The Role of Interleukin-32 in the Pathogenesis and Exacerbation of Bronchial Asthma

**By Jae-Woo Kwon**

**A thesis submitted in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Medicine (Immunology) at Seoul National University College of Medicine**

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## Abstract

# The Role of Interleukin-32 in the Pathogenesis and Exacerbation of Bronchial Asthma

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**Background**

Innate immunity and infection are important in the development and exacerbation of asthma. Interleukin-32 (IL-32), a pro-inflammatory cytokine involved with innate immunity against various infectious stimuli, is involved in various chronic inflammatory diseases such as rheumatoid arthritis, Crohn′s disease, and chronic obstructive pulmonary disease (COPD). This study was conducted to evaluate the role of IL-32 in asthma.

**Methods**

The role of IL-32γ in the airway was investigated in human bronchial

epithelial cells (BEAS2B) stimulated with common asthma aggravating factors such as NOD ligands, dsRNA, lipopolysaccharide (LPS), and allergen (Der p) from house dust mites. IL-32γ was measured in the plasma of patients with asthma (n = 103) and healthy controls (n = 51), and then determined in induced sputum supernatant of patients with asthma (n = 89). Relationships between IL-32γ, airway obstruction ($FEV_1$), inflammation (neutrophil and eosinophil % of the airway) and exacerbation frequency were evaluated.

**Results**

In in vitro study, IL-32γ exhibited synergistic effects with NOD 1 ligand and dsRNA on the induction of IL-6 from BEAS2B cells, but not by LPS and Der p. Plasma IL-32γ was detected in 95% (98/103) of patients with asthma and the level of IL-32γ was higher in asthmatic patients than in healthy controls. In the analysis of induced sputum, IL-32γ was detected in the sputum of 25 of 89 (28.1%) patients with asthma, and the asthma exacerbation rate was significantly higher in this group (n = 25) than in the IL-32-negative group (n = 64) ($p = 0.03$).

**Conclusion**

This study suggests that IL-32γ in the airway could increase inflammatory response to infectious stimuli and there could be an important role of IL-32 in subtype of asthma characterized by frequent exacerbations.

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**Keywords**: IL-32, asthma, exacerbation

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# Introduction

Asthma is a chronic inflammatory airway disease that presents with typical symptoms such as episodic and fluctuating dyspnea, wheezing, and cough.[1)] Asthmatic airway inflammation is generally characterized by the allergic pathology of Th2 inflammation. However, recent studies have suggested that there are heterogeneity among clinical phenotypes of asthma and possibly among underlying disease pathophysiology of asthma, so called 'endotype'.[2)] Many studies have identified an important role of innate immunity in asthma pathogenesis including airway epithelial-derived cytokines.[3)] Such cytokines have been described as "epithelial-derived alarmins" that activate the innate immunity and could induce Th2-type inflammation, even without adaptive immunity.[4)] On the other hand, respiratory pathogens are not only the common causes of asthma exacerbations but also suggested as risk factor of asthma development.[5)]

Interleukin-32 (IL-32) is a pro-inflammatory cytokine involved with innate immunity against various infectious stimuli and proposed to function as an alarmin.[6,7)] IL-32 is produced by epithelial cell lines, T lymphocytes, natural killer cells, and monocytes. IFN-γ, IL-12 and IL-18 have been shown to induce IL-32, whereas IL-32 can provoke the release of TNF-α and IL-1β.[8)] IL-32 is involved in chronic inflammatory diseases including rheumatoid arthritis, Crohn's disease, and COPD and is correlated with disease severity.[9)]

IL-32 expression is increased in lung tissue of patients with COPD,[10,11] and negatively correlated with $FEV_1$.[10] In patients with Crohn's disease, IL-32 expression is increased in intestinal epithelium and the relationship between IL-32 and the NOD pathway is critical in development of Crohn's disease in some cases.[12] However, the exact function of IL-32 remains controversial in asthma. Meyer N et al.[13,14] found a more frequent presence and increased levels of plasma IL-32 in asthmatic patients and a correlation with response to asthma treatments. On the contrary, Bang et al.[15] reported decreased levels of IL-32γ in both serum and sputum of asthmatic patients compared to healthy controls and demonstrated the protective role against asthma development using IL-32γ transgenic mice. In addition, clinical implication of IL-32 has not been demonstrated in terms of asthma exacerbations up to date.

This prompted us to evaluate the relationship between IL-32 and asthma triggering factors in the airway using bronchial epithelial cells and various infectious stimuli. We measured the IL-32 levels in plasma and supernatants of induced sputum of patients with asthma and analyzed the relation with asthma exacerbations and inflammation profiles of sputum. We also measured IL-33 which is one of the above-mentioned "epithelial-derived alarmins" and has been studied extensively for its role in asthma pathogenesis, but never with IL-32.[4,16] IL-33 can stimulate basophils and mast cells to produce Th2 cytokines without allergen and IgE antibodies, and stimulates ILC2s to produce large

amounts of IL-5, IL-9, and IL-13.[17] And thus IL-33 is suspected to induce allergic inflammation from innate immunity related with infectious stimuli although relationships between clinical findings of asthma and IL-33 still have not been revealed clearly. In order to understand the role of innate immunity in asthma, we decided it would be helpful to evaluate both IL-32 and IL-33 in bronchial epithelial cells, as well as in plasma and sputum samples from asthmatic patients.

# Methods

## 1. In vitro study

Possible role of IL-32 in asthma exacerbation were evaluated by in vitro study.

### *Materials*

NOD1 ligand iE-DAP and NOD2 ligand N-acetylmuramyl-L-alanyl-D-iso-glutamine (muramyl dipeptide/MDP) were purchased from Invivogen (San Diego, USA). Recombinant human IL-32γ was purchased from R&D Systems. Lipopolysaccharide (LPS) and polyinosinic-polycytidylic acid (poly I:C) were purchased from Sigma-Aldrich. Allergen extract (Der p) from house dust mites was provided by Arthropods of Medical Importance Resource Bank, Institute of Tropical Medicine, College of Medicine, Yonsei University, Seoul, Korea

### *Cell culture*

BEAS-2B cell line, a SV40-transformed human bronchial epithelial cell line, was purchased from the ATCC (Manassas, VA, USA). The cells were grown in LHC-9 medium (complete culture medium) in a humidified atmosphere at 37℃ with 5% CO2. The medium was refreshed every 2–3 days. BEAS-2B cells were plated into 12-well coated-culture dishes ($5 \times 10^5$ cells/well).

### *Measurement of cytokine levels by ELISA and Real-time quantitative PCR*

After exposing BEAS-2B cells to poly I:C (50 μg/ml) for 24 h, the quantitative

expressions of human b-actin, IL-32γ were detected by Real-time PCR. After exposing BEAS-2B cells to iE-DAP (1–10 μg/ml), MDP (1-10 μg/ml), poly I:C (0.1 μg/ml), LPS (0.1 μg/ml), and Der p (5-20 ug/ml) with or without rhIL-32 (10-50 ng/ml) for 24 h, the cell culture supernatants were collected and stored at −70℃ until cytokine analysis. Concentrations of IL-6 and IL-8 in cell culture supernatants were determined by ELISA (ebioscience, USA), according to the manufacturer's manual. Total RNA of cells was extracted using TRI-Reagent (Ambion Inc., Austin, TX, USA). Extracted RNA was then reverse-transcribed into complementary DNA using cDNA synthesis kit (Applied Biosystems, Foster City, CA, USA). The quantitative expressions of human b-actin, IL-32γ and IL-33 were performed using Universal SYBR Green Master (Applied Biosystems) with primers of IL-32γ: forward 5′-GACAGTGGCGGCTTATTATGAG-3′ and reverse 5′-CCCAGATCACGAAGGAGTCTATT -3′; and IL-33: forward 5′-GTGACGGTGTTGATGGTAAGAT-3′ and reverse 5′-AGCTCCACAGAGTGTTCCTTG-3′. Expression of each gene within each sample was normalized against β-actin and expressed relative to the control sample using the formula 2 − (ΔΔCt), in which ΔΔCt = (Ct mRNA − Ct b-actin).

## 2. IL-32 in patients with asthma

***Separate group of patients for each sample (plasma and induced donated***

***sputum)***

Based on the findings from in vitro study, IL-32 and IL-33 were evaluated in plasma and sputum of patients with asthma. Plasma and induced sputum samples for normal controls and patient cohort for asthma were obtained from Biobank at Soonchunhyang University Bucheon Hospital. Separate groups of patients donated plasma or sputum, except for a few patients who donated both plasma and sputum samples. The levels of IL-32, IL-33, and IL-1β were measured in plasma of normal controls and in patients with asthma. IL-32 and IL-33 levels were then measured in the supernatants of the induced sputum of asthma patients. The relationship among these cytokines, severity of asthma and the clinical characteristics of asthmatic patients, such as inflammatory cell profiles in induced sputum and lung function tests, were evaluated.

**1) Study subjects**

***Patients with asthma***

Asthma was diagnosed by physicians on the basis of the Global Initiative for Asthma (GINA) guideline.[18)] The diagnosis was supported by one or more of the following criteria: an increase in forced expiratory volume in 1 min ($FEV_1$) > 12% and an increase > 200 mL after inhalation of 400 μg albuterol, a 20% reduction in $FEV_1$ in response to a provocative concentration of inhaled methacholine (PC20) < 10 mg/mL, or an increase in $FEV_1$ > 20% after 2

weeks of treatment with systemic or inhaled corticosteroids. The patients were recruited from a tertiary hospital. Current smokers and ex-smokers with > 10 pack-years were excluded.

Patients defined as having stable status of asthma exhibited no asthma exacerbation within 4 weeks prior to sputum collection. Steroid-non-treated patients were defined as who did not use systemic or inhaled corticosteroids for ≥ 4 weeks before sputum collection. Exacerbation of asthma was defined as one or more episodes of short-term treatment with a systemic corticosteroid to manage increased asthmatic symptoms or episodes characterized by increased dyspnea, cough, wheezing, or chest tightness with a $FEV_1$<80% of the patient's personal best.[18,19] Demographic information, including enrollment age, sex, body mass index (BMI), tobacco consumption, age of asthma onset, and asthma duration, was collected at the baseline visit. All patients underwent a standardized assessment that included induced sputum analysis, complete blood cell counts with differential counts, serum total IgE levels, chest radiography, spirometry, and allergy skin prick tests with 24 common inhalant allergens (Bencard Co., Brentford, UK). Atopy was defined using the skin prick tests; positivity was defined as a mean wheal diameter ≥ 3 mm over that of the saline control. Patients were categorized according to the inflammatory subtype of their sputum sample:[20] neutrophil dominant (neutrophils ≥ 70%, eosinophils < 3%, n = 30), eosinophil dominant

(neutrophils < 70%, eosinophils ≥ 3%, n = 32), co-dominant (neutrophils ≥ 70%, eosinophils ≥ 3%, n = 14), and pauci-granulocytic (neutrophils < 70%, eosinophils < 3%, n = 13). Patients were examined in the outpatient clinic every 2 months, and their diary cards recording their asthma episodes were collected.

***Normal controls***

Control subjects were selected by a criterion of normal spirometry values; subjects who had a history of asthma or COPD were excluded. Demographic characteristics, smoking status, pre-bronchodilator lung function tests, analyses of induced-sputum specimens, and complete blood cell count with differential counts were collected. Serum levels of total IgE were evaluated only in control subjects.

***Biobank***

Informed written consent including voluntary donation of their blood and sputum to the Biobank at Soonchunhyang University Bucheon Hospital were obtained from all participants, using a protocol approved by the ethics committee of the hospital (SCHGM 2014-16). The samples were obtained after permission by the distribution committee of Soonchunhyang University Hospital Biobank (schbc-biobank-2014-009-01). Sputum was induced using isotonic saline that contained a short-acting bronchodilator, and samples were

processed as previously described.[21] These studies were approved by the Kangwon National University Hospital Institutional Review Board (KNUH-2015-02-004-001).

**2) Sample preparation and measurement of cytokines**

***Procedure of sputum induction and preparation***

Sputum was induced using isotonic saline containing a short-acting bronchodilator. The samples were processed as previously described [21]. Briefly, all samples with visibly higher solidity were carefully selected and placed in a pre-weighed Eppendorf tube to which eight volumes of 0.05% dithiothreitol (Sputolysin; Calbiochem Corp., San Diego, CA, USA) in Dulbecco's phosphate-buffered saline (PBS) were added. Protease inhibitor (0.1 M methylene-diamine-tetra-acetic acid and 2 mg phenyl-methyl-sulfonyl-fluoride/mL) were then added to the homogenized sputum at a ratio (v/v) of 1:100. Total cell counts were determined using a hemocytometer. Sputum cells were collected by cytocentrifugation, and 500 cells were examined after staining with Diff-Quick (American Scientific Products, Chicago, IL, USA). Samples that contained > 10% squamous epithelial cells were excluded from the study. The remainder of the homogenized sputum sample was centrifuged at 1,000 g for 5 min; the supernatant was collected and stored at −70℃ for subsequent protein analyses.

***Quantitative measurement of cytokines using ELISA***

IL-32γ was measured in duplicate using a sandwich ELISA kit (YbdY Biotechnology, Seoul, Korea) according to the manufacturer's instructions. The limit of detection was 0.082 ng/mL. Values below this level were scored as 0 ng/mL for statistical analysis. IL-33 was measured in duplicate using a sandwich ELISA kit (YbdY Biotechnology, Seoul, Korea) according to the manufacturer's instructions. Mean values are presented. The inter- and intra-assay variations were less than 15%. The lower limit of detection was 0.082 ng/mL, any values below this level were scored as 0 ng/mL on statistical analysis.

## 3. Statistical analysis

The normality of distribution was evaluated using the Shapiro–Wilk test. Nonparametric variables and parametric variables were compared using Mann–Whitney U-tests or t-tests, respectively. Categorical values were compared using a $\chi^2$ test. Correlations between IL-32 levels and sputum cell percentages and other parameters were determined using Spearman's rank correlation coefficient analysis. The data are presented as the median and 25% and 75% quartiles for skewed variables and as the mean ± SEM for variables with a normal distribution. For plasma cytokine analysis of asthmatic patients and normal controls, baseline characteristics are presented as means and standard deviations (SD) for continuous variables and as relative frequencies

for categorical variables. Among the variables, BMI, FVC, and $FEV_1$ followed normal distribution while the others did not. Correlations among the levels of cytokines, sputum cell counts, and the variables of interest that did not follow a normal distribution were analyzed by Spearman's rank correlation coefficient analysis. Comparisons of nonparametric variables and parametric variables were performed using Kruskal-Wallis tests and ANOVA, respectively, and then post hoc analyses using Mann-Whitney U-tests or t-tests were performed. A general linear model was used to compare plasma level of IL-32 between asthma and normal controls after adjustment for $FEV_1$. For in vitro study, All data were expressed as mean ± SD. from three independent experiments. The statistical significance of differences was determined by unpaired t-tests. A value of $p < 0.05$ was considered significant. All statistical analyses were performed using SPSS ver. 20.0 (SPSS Inc., Chicago, IL).

# Results

## 1. In vitro Study

### Induction of IL-32 in BEAS2B cells

BEAS2B cells were incubated with various stimuli that are suggested as asthma aggravating factors in order to investigate IL-32 expression. After 24 hours of stimulation with poly I:C, IL-32γ mRNA expression increased more than 100 fold (Figure 1).

### Effects of IL-32γ and asthma aggravating factors for the induction of cytokines from bronchial epithelial cells

The effects of IL-32γ on the induction of cytokines from bronchial epithelial cells by various triggering factors of asthma exacerbations were evaluated. BEAS2B cells were treated with NOD1, 2 ligands and IL-32γ. Stimulation with high dose NOD1, 2 ligands (50 ng/ml) with IL-32 (50 ng/ml) demonstrated additive effects for inducing IL-6 and IL-8 from bronchial epithelial cells. Figure 2 shows that IL-32γ (50 ng/ml) could induce IL-6 and IL-8, and could exhibit an additive effect with NOD1 ligand iE-DAP (10 μg/ml) on the expression of IL-6 and IL-8 (Figure 8). The expression of IL-33 mRNA was not affected significantly by IL-32γ and NOD 1, 2 ligands (Figure 2C).

Further studies using low dose NOD ligands showed that IL-32γ exhibited

synergistic effects with low dose NOD1 ligands iE-DAP (1 μg/ml) on induction of IL-6 (Figure 3). Production of IL-8 in BEAS2B was not affected by co-stimulation with IL-32γ and low dose NOD ligands. The expression of IL-32 mRNA was very low in BEAS2B and there seemed to be no significant changes in IL-32 mRNA by NOD ligands and IL-32 stimulation. Co-stimulation with low dose NOD2 ligands MDP and IL-32γ did not increase IL-6, IL-8, and IL-33 mRNA in BEAS2B (Figure 4). IL-32γ mRNA expression was not changed by stimulation with NOD1,2 ligands and IL-32 (Figure 5).

After 24 hours of stimulation with IL-32γ and various stimuli such as Poly I:C (TLR 3 ligand), LPS (TLR4 ligand), and Der p (allergen from house dust mites), which are all common factors for asthma exacerbations, IL-32γ exhibited synergistic effects with Poly I:C on the induction of IL-6 and IL-8 (Figure 6). Figure 7 shows that LPS induced IL-6 and IL-8 from bronchial epithelial cells but there were no additional effects or co-stimulation with IL-32γ. Figure 8 shows that the effects of Der p and IL-32γ stimulation were unclear on the induction of IL-6, IL-8, and IL-33.

## 2. Plasma IL-32 in the patients with Asthma

***Baseline characteristics***

IL-32 and IL-33 were quantified in the plasma of patients with asthma (n = 103) and healthy controls (n = 51) using ELISA. The characteristics of the

study population are presented in Table 1.There were differences in age, BMI, $FEV_1$ (% pred.), FVC (% pred.), $FEV_1$/FVC, smoking history, serum total IgE, and induced sputum eosinophilia between the two groups.

***Plasma level of IL-32 and IL-33 in patients with asthma***

Plasma levels of IL-32 were higher in patients with asthma compared with normal control (Figure 9A). Plasma levels of IL-33 were also higher in patients with asthma compared with normal control (Figure 9B). Analysis after transforming the crude values into log values provided the same results for plasma IL-32 and IL-33 between the two groups. Plasma IL-32 was detected in 95.1% (98/103) of patients with asthma and plasma IL-33 was detected in 80% (82/103) of asthmatic patients. Meanwhile, the detection rate of plasma IL-32 and IL-33 in healthy controls was 94.1% (48/51) and 49.0% (25/51), respectively. The detection rate of IL-33 was different between the two groups ($p < 0.0001$). There was positive correlation between plasma levels of IL-32 and IL-33 in asthmatic patients and normal controls ($r = 0.720$, $p < 0.0001$, and $r = 0.784$, $p < 0.0001$; Figure 10).

Plasma levels of IL-33 showed negative correlation with $FEV_1$ (% pred.) and positive correlation with macrophages percentage in induced sputum ($r = -0.215$, $p = 0.029$ and $r = 0.363$, $p = 0.008$, respectively). IL-33 was negatively related with BMI ($r = -0.157$, $p = 0.009$). Plasma levels of IL-32 were negatively related with BMI and age ($r = -0.230$, $p = 0.02$ and $r = -0.395$, $p <$

0.0001), and still showed negative correlation with age after adjustments for BMI in linear regression ($p < 0.0001$). However, plasma levels of IL-32 and IL-33 showed no relation with serum levels of total IgE and the presence of atopy. Blood eosinophil count, inflammatory profiles in induced sputum, and annual exacerbation rates were not related with IL-32 nor IL-33. On the other hand, IL-1β was rarely detected in plasma of normal control (4/51) or asthmatic patients (9/103).

## 3. IL-32 in the Induced Sputum of Patients with Asthma

### *Clinical characteristics of the study subjects*

A total of 89 patients with asthma were enrolled with induced sputum samples. The characteristics of the study population are presented in Table 2. The median follow-up time was 4.08 years; the interquartile range was 1.05 – 8.41 years after the baseline visit.

### *IL-32 protein levels in the sputum of asthmatics*

The level of sputum IL-32 was 0.77 (1.23) ng/ml, mean (standard deviation). Sputum IL-32 was detected in 25 of 89 asthma patients (28.1%). Sputum IL-32 levels correlated negatively with $FEV_1$ (% pred.) and FVC (% pred.) ($r = -0.312$, $p = 0.003$ and $r = -0.316$, $p = 0.003$, respectively (Figure 11A). In the subgroup analysis of the 25 patients with detectable sputum IL-32, the levels correlated inversely with $FEV_1$ (% pred.) and $FEV_1$/FVC ($r = -0.463$, $p =$

0.020 and r = −0.520, $p = 0.008$, respectively).

Sputum IL-32 levels also correlated positively with the annual exacerbation rate (r = 0.261, $p = 0.014$; Figure 5B) and the correlation remained significant after adjustment for $FEV_1$ (% pred.) ($p = 0.041$). In contrast, sputum IL-32 levels did not correlate with either the $PC_{20}$ or the total serum IgE level. There was also no correlation between either sputum neutrophilia or eosinophilia and the sputum IL-32 level (r= −0.101, $p = 0.681$ and r = 0.054, $p = 0.828$, respectively). Steroid-treated and steroid-non-treated patients did not differ in their sputum IL-32 levels or in the results of their lung function tests.

***Comparison of the clinical and laboratory parameters of patients with detectable and undetectable sputum IL-32***

Patients with detectable sputum IL-32 levels (n = 25) had a significantly lower age of asthma onset and significantly lower $FEV_1$ (% pred.) and FVC (% pred.) values compared with those with undetectable sputum IL-32 (n = 64; $p = 0.034$, 0.01, and 0.011, respectively; Table 3). The asthma exacerbation rate was significantly higher in patients with detectable sputum IL-32 than in those with non-detectable sputum IL-32 (per 100 person years, 86.8 vs. 16.2, $p = 0.030$; Figure 12).

***IL-33 protein levels in the sputum of asthmatics***

Sputum IL-33 was detected in 79.8% (71/89) of patients with asthma. The

median level (interquartile range) of sputum IL-33 was 0.32 (0.08-0.60) ng/ml. Sputum IL-33 showed no correlations with sputum IL-32, $FEV_1$ (% pred.), FVC (% pred.), blood eosinophilia, inflammatory profiles of induced sputum, serum total IgE, or $PC_{20}$. However, patients with detectable sputum IL-33 (n = 71) showed higher percentage of eosinophils [median (interquartile range): 6.0% (0.0–24.3) vs. 0.0% (0.0–2.6) , $p$ = 0.003] and lower percentage of neutrophils [61.3% (40.3–81.8) vs. 87.3% (69.9–93.9), $p$ = 0.004] in the induced sputum than patients with undetectable sputum IL-33 (n = 18).

## Discussion

When studied in vitro, IL-32γ exhibited synergistic effects with NOD 1 ligand and dsRNA on the induction of IL-6 and IL-8 from bronchial epithelial cells. IL-32γ was detected in the sputum of less than one-third of the patients with asthma. Asthmatic patients with detectable IL-32γ had a lower $FEV_1$ and a higher rate of annual asthma exacerbation than patients who were negative for sputum IL-32γ. These findings suggest a relationship between IL-32γ in the airways and the severity of asthma in terms of airflow limitation and exacerbation.

This study found that IL-32 was detected in the airway among the subpopulation of asthmatic patients who showed more frequent exacerbations. In vitro results suggested that the presence of IL-32 could enhance the inflammatory response to subclinical infectious stimuli in bronchial epithelial cells. Other in vitro studies suggest IL-32 as a possible regulator of innate immune responses to oxidative stress and various infectious stimuli.[6,7,22] Viral stimulation and oxidative stress-induced IL-32 in human bronchial epithelial cells,[23,24] and iNOS activation by viral infection in lung epithelial cells is closely related with IL-32.[22] Synergism between IL-32 and the NODs pathway for inflammatory cytokine production in mucosal immunity and synergistic effects of IL-32 and NOD ligand on the activation of human eosinophils via interaction with bronchial epithelial cells has been previously

reported.[8,12,25] Viral infections such as respiratory syncytial virus and human rhinovirus have been implicated in asthma exacerbation and possibly in asthma inception in children.[5] Bacterial Infections also appear to play a role in the induction and exacerbation of asthma in both children and adults.[26] Recent studies confirm the existence of an infectious asthma etiology mediated by *Chlamydia pneumoniae* and possibly by other viral, bacterial and fungal microbes.[26] NOD1 and NOD2 are intracellular pattern-recognition receptors for bacterial molecules. Thus the presence of IL-32 in the airway could be related to chronic infectious stimuli or oxidative stress, and pre-existing IL-32 may cause synergistic immune response with subclinical infectious stimuli, which may result in more frequent exacerbations of asthma. This study demonstrated that low dose NOD1 ligand stimulation with IL-32 could induce synergistic induction of IL-6 and possibly IL-33 in BEAS2B cells. Furthermore, poly I:C, which usually mimics viral stimulation, could induce the mRNA expression of IL-32 in BEAS2B cells and showed synergistic effects with IL-32 to produce IL-6 and IL-8. Recent studies have reported IL-6 inflammation may be associated with asthma severity in a subset of asthma patients,[27] as well as IL-8 which is one of well-known pro-inflammatory cytokines.

Sputum IL-32 was detectable only in 28.1% (25/89) of the asthma patients. Detection rates of sputum IL-32 in this study are similar those of Meyer et al.,

in which 8 of 22 (36.4%) sputum samples from asthmatic patients were IL-32-positive, compared with only 1 of 9 (11.1%) induced sputum samples from healthy control subjects.[14] Although the cellular sources of sputum IL-32γ were not determined in the current study, IL-32 has been shown to originate from apoptotic bronchial epithelial cells [28] and immune cells, such as macrophages, dendritic cells, and T cells, all of which are involved in the inflammatory process of asthma [10,29,30] Viral stimulation and oxidative stress induce IL-32 release by human bronchial epithelial cells [23,24]. IL-32 is also expressed in lymphoid follicles (LF) [11], and both IL-32 and the number of LFs are correlated with the number of B cells and CD4 T cells in the lungs of smokers and COPD patients. In a microbiome analysis, bacterial DNA was identified in the lungs of patients with severe COPD, where it may promote the formation of tertiary LFs, particularly in the small airways.[31-34] IL-32 and TNF-α are co-expressed by macrophages in the lung tissue of COPD patients.[10,11] IL-32-positive macrophage levels are significantly higher in COPD patients, especially in those with alpha-1 antitrypsin (AAT)-deficient emphysema. Interestingly, AAT inhibits IL-32 activation,[35] and asthma symptoms are common in AAT-deficient patients, with or without COPD.[36] Furthermore, AAT deficiency itself predisposes to airway hyper-responsiveness, an essential component of reversible airflow obstruction. Thus, the blunted local production and functional inactivation of AAT might lead to elevated IL-32 levels in the sputum of asthma patients.

There were no differences in the detection rates and sputum IL-32 levels according to corticosteroid treatments in our study. This result suggests that IL-32 levels are not affected by inhaled corticosteroid and is consistent with previous reports that sputum IL-32 is not reduced by inhaled corticosteroids/long-acting β-agonist treatment in COPD patients and that inhaled corticosteroids did not alter IL-32 levels in the serum of asthmatic patients.[14,37] Although further studies are needed, sputum IL-32 might be an indicator of an asthma subtype with frequent asthma exacerbation, which could be measured in stable status of asthma and not affected by corticosteroid treatments. On the other hand, IL-32 expression correlates with the number of neutrophils infiltrating the alveolar walls and with the degree of airflow obstruction in COPD.[10] Neutrophil-derived proteinase 3 (PR3), a protease expressed mainly in neutrophil granulocytes, binds to IL-32α to enhance its biological activities. Limited cleavage by PR3 of IL-32 induces both macrophage inflammatory protein 2 and IL-8.[38] Our analysis of neutrophil cell numbers and fractions failed to demonstrate a correlation with sputum IL-32 levels ($r = -0.101$, $p = 0.681$). Although we did not measure neutrophil activity including that of PR3, these results suggest that neutrophil infiltration has no effect on IL-32 levels in sputum.

For allergic diseases, increased expressions of IL-32 were observed in nasal mucosa of patients with allergic rhinitis and skin lesions of atopic

dermatitis.[13,39] IL-32 is robustly expressed in keratinocytes of atopic dermatitis and the extent of expression was correlated with severity of skin lesions.[13] In patients with allergic rhinitis,[39] IL-32 up-regulation in nasal mucosa is associated with increased inflammatory cytokine production including IL-1β, IL-18 and GM-CSF. Taken together, IL-32 is elevated in target organs of allergic diseases and may be related with disease severity, but relation with eosinophilic inflammation is not clear. There was no correlation between serum levels of IL-32 and IgE in patients with atopic dermatitis and in vitro study showed IL-32 is expressed in human keratinocytes upon stimulation with IFN-γ, TNF-α, and Th1 cells in contrast to Th2, Treg, and Th17 cells, which showed no effect.[13] These findings suggest that expression of IL-32 could be elevated in asthma but via different pathways from Th2 inflammation. The diverse manifestations induced by IL-32 have also been demonstrated in several animal models of allergic diseases. In an allergic rhinitis animal model using BALB/C, IL-32 significantly increased IgE and Th2 inflammation and is essential for production of inflammatory cytokines by eosinophils stimulated with GM-CSF [39]. On the contrary, high levels of IL-32γ in IL-32γ transgenic mice on a C57BL/6 background suppressed the development of chronic allergic airway inflammation.[15] The different methods of sensitization and challenges using ovalbumin/aluminum hydroxide and OVA/protease (Aspergillus melleus protease) in different mice strains may contribute to the diverse manifestations of IL-32 as observed in human allergic

diseases.[13-15,39] As for asthma, Meyer et al.[14] showed the serum level of IL-32 is correlated positively with serum TNF-α and IFN-γ levels in sera from asthmatic patients with detectable serum IL-32, while IL-17 and IL-13 levels were also elevated in those patients. For the studies using the asthma animal model, IL-32 did exhibit protective effects against asthmatic inflammation in IL-32 transgenic mice[15] and in vitro examination suggested that IL-32 might be an important inhibitor of airway remodeling by the reduction of angiogenesis in asthmatics.[14] Although ascertaining the exact role of IL-32 in asthma pathogenesis requires further study, the current results suggest that IL-32 might participate in the increased risk for asthma exacerbations possibly by enhancing the inflammatory response to infectious stimuli.

Heterogeneity of asthma has been suggested based on clinical characteristics and clinical course. Airway epithelial and immune cells have been prominently implicated in asthma pathogenesis as part of an effort to identify subgroups sharing an underlying disease pathobiology, 'endotype' of asthma.[40] Several studies using whole-genome microarray expression profiling of airway epithelial brushings showed that only 50% of asthmatic patients exhibit high expression of genes related with Th2 cytokines, termed 'Th2-high', while the others clustered into non-Th2 asthmatic patients which usually show neutrophilic inflammation and airway remodeling.[41] Wenzel SE suggested that patients with non-Th2 asthma show less airway obstruction and

hyperreactivity than people with Th2-high asthma, and many of them have mild to moderate adult-onset asthma without history of childhood allergic features.[42] Non-Th2 asthma is also suspected to include various phenotypes such as very late–onset, obesity-associated asthma, smoking-related asthma, neutrophilic asthma, and asthma in which affected individuals show little inflammation.[42] In contrast with Th2-high asthma with many biomarkers for Th2-inflammation, there are no molecules or cytokines to represent the pathophysiology of non-Th2 asthma. However a recent study reported the association between IL-6 concentrations and asthma severity in a subset of asthma patients characterized by non-Th2 severe asthma; IL-6 is a biomarker of systemic inflammation and metabolic dysfunction.[27] In the current study, asthmatic patients with sputum IL-32 showed relatively late-onset and less inflammation in induced sputum than generally expected in patients with Th2-high asthma, and showed low $FEV_1$ and frequent asthma exacerbations regardless of airway eosinophilia. Furthermore IL-32 increased IL-6 levels in response to infectious stimuli in airway epithelial cells. These findings might suggest a possible role of IL-32 in pathogenesis of non-Th2 asthma based on innate immunity with infectious stimuli, although further studies are needed.

Plasma levels of IL-32 were higher in patients with asthma compared to controls, but there was no relation with clinical characteristics of asthma including asthma exacerbations and inflammation profiles of induced sputum.

These findings are consistent with previous findings.[13,14] Meyer et al.[14] reported that serum IL-32α and IL-32β were detected more frequently in asthmatics (40%, 40/99) than in healthy controls (6.3%, 1/16), and that there were no differences in smoking behavior, serum IgE levels, age of onset, or previous exacerbations within 1 year according to the presence of serum IL-32 in patients with asthma. Regardless, in the current study plasma levels of IL-32γ were higher in patients with asthma than normal controls regardless of $FEV_1$ but showed no relations with eosinophilic inflammation or serum level of total IgE in asthma patients. On the other hand, Meyer et al.[14] reported that presence of serum IL-32 was related with response to asthma treatments in terms of $FEV_1$. In fact, the group with serum IL-32 seemed to show lower $FEV_1$ in that study, possibly making them more likely to show a better response to asthma treatment.[13] Although there were no data about response to asthma treatment in the current study, it seemed that the high IL-32 group in both study showed more severe asthma in terms of lung function deficit.

Plasma levels of both IL-32 and IL-33 were higher in patients with asthma than normal controls. However, in contrast with plasma levels of IL-32 which showed no relations with clinical characteristics of asthma, plasma level of IL-33 were negatively correlated with $FEV_1$ (% pred.), although there were no relationships with serum IgE level and atopy. These results were consistent with previous studies which reported that IL-33 is expressed more abundantly

in the lung epithelial cells of asthmatic patients than healthy controls,[43] and there was no significant association between the total serum IgE level and serum IL-33 level.[44] The relationship between sputum IL-33 and clinical characteristics of asthma have been inconsistent in previous studies. Although IL-33 is regarded as one of key cytokines in pathogenesis of asthma related with innate immunity and associated with asthma severity,[45,46] there were no correlations between sputum IL-33 and clinical findings of asthma such as lung function, asthma exacerbations, and inflammatory profiles of induced sputum. These findings suggest that there may be many other factors that contribute to asthma pathogenesis. However, it could also be possible that the role of IL-33 is persistent in maintaining asthma pathogenesis and the role of IL-32 may be more prominent in acute exacerbation of asthma. And it was an interesting finding that there was positive correlation between plasma level of IL-32 and IL-33 in asthmatic patients and healthy controls. This is the first study to report the correlation between plasma IL-32 and IL-33.

Several limitations of this study should be discussed. First, there were no serial measurements of sputum IL-32γ or longitudinal data such as changes of lung function, symptom frequency and medication. Further study using serial measurements of sputum IL-32 is needed to evaluate the consistency of sputum IL-32 and to know changes of sputum IL-32 during asthma exacerbations. In addition, study populations were different between the

patient group with sputum IL-32 and IL-33 measurements and patient group with plasma IL-32 and IL-33 measurements. It would be useful to understand the role of cytokines in asthma if we could evaluate the IL-32 and IL-33 both in airway and plasma simultaneously in same study population. Second, most of enrolled patients with asthma did not have severe asthma; patients with > 1 annual exacerbation were only 8 among 89. So it was not possible to know the role of sputum IL-32 in severe asthma requiring multiple controller medications including high dose inhaled steroid or systemic corticosteroids and there could be many other factors in the risk of asthma exacerbation including IL-32. Furthermore, the characteristics of patients in this study may not be representative of asthmatics in the general population because our patients had an older age of asthma onset and a shorter duration of asthma, the so called “late-onset” asthma subtype. Finally, there were no measurements for Th2 and non-Th2 cytokines in plasma and sputum of patients with asthma. To understand the role of IL-32 in asthma pathogenesis and to know the position of IL-32 among asthma heterogeneity, other cytokines including the Th2 cytokines such as IL-4, IL-5, and IL-13, and non-Th2 cytokines such as IFN-γ, IL-6, IL-8, and CXC chemokines should be evaluated in a larger study population, even although there are a very limited number of biomarkers for non-Th2 asthma.

In summary, IL-32γ exhibited synergistic effects with NOD 1 ligand and

dsRNA to induce IL-6 and IL-8 in bronchial epithelial cells in vitro. IL-32γ was detected in the sputum of a subset of the asthma patients, and this subset showed higher rates of annual asthma exacerbation than patients without detectable sputum IL-32γ. Although there seem to be many other factors related to asthma exacerbation and further studies are needed, our current study suggests that IL-32γ in the airway could increase the inflammatory response to infectious stimuli and indicates there could be an important role of IL-32 in the subtype of asthma characterized by frequent exacerbations.

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**Table 1.** Characteristics of study population

| Characteristics | Asthma | Normal control | p-value |
|---|---|---|---|
| Subjects (n) | 103 | 51 | |
| Male/Female | 29 / 74 | 39 / 12 | |
| Age (range) | 50.03 (17-80) | 64.60 (40-86) | < 0.0001 |
| Age of onset | 42.80 ± 16.19 | - | |
| Duration of asthma (years) | 7.24 ± 9.30 | - | |
| Smoking status (NS/ES/SM) | 85 / 13 / 5 | 17 / 10 / 24 | < 0.0001 |
| Amount of smoking (pack-year) | 3.81 ± 10.71 | 19.90 ± 23.39 | < 0.0001 |
| FVC (% pred.) | 71.01 ± 16.88 | 99.24 ± 12.91 | < 0.0001 |
| $FEV_1$ (% pred.) | 64.68 ± 22.03 | 111.59 ± 13.75 | < 0.0001 |
| $FEV_1$/FVC | 67.80 ± 12.54 | 81.20 ± 5.62 | < 0.0001 |
| BMI | 23.35 ± 3.24 | 23.47 ± 4.34 | 0.874 |
| $FEV_1$ changes (%) post-BDR | 12.94 ± 11.51 | - | |

| | | | |
|---|---|---|---|
| $FEV_1$ changes (L) post-BDR | 0.19 ± 0.18 | - | |
| $PC_{20}$ | 6.33 ± 9.16 | - | |
| Serum total IgE | 442.83 ± 859.72 | 150.70 ± 209.28 | 0.004 |
| Exacerbation (Y/N/ND) | 65 / 18 / 11 | - | |
| Induced sputum* | | | |
| Neutrophils count | 234.67 ± 128.88 | 198.44 ± 140.15 | 0.298 |
| Neutrophils (%) | 62.28 ± 32.89 | 53.99 ± 37.10 | 0.298 |
| Eosinophil count | 107.02 ± 125.43 | 0.88 ± 2.31 | < 0.0001 |
| Eosinophils (%) | 27.36 ± 32.53 | 0.22 ± 0.58 | < 0.0001 |

Mean ± SD

* Induced sputum data of normal controls obtained from 16 subjects

NS, non-smoker; ES, ex-smoker; SM, current smoker; BDR, bronchodilator response, $PC_{20}$, 20% reduction in $FEV_1$ in response to a provocative concentration of inhaled methacholine

**Table 2.** Characteristics and interleukin (IL)-32 levels in the sputum of asthma patients

| Characteristic | Patients with asthma |
|---|---|
| Subjects (n) | 89 |
| Male/Female | 20 / 69 |
| Age (years) | 51.5 (37.9-61.4) |
| Age of asthma onset (years) | 46.5 (34.2-57.7) |
| Total follow-up periods (years) | 4.1 (1.1-8.4) |
| Smoking status (NS/ES) | 79 / 10 |
| BMI ($kg/m^2$) | 24.6 (21.5-26.7) |
| Atopy (N/Y/ND) | 50 / 35 / 4 |
| Steroid treatment (Y / N) | 11 / 78 |
| Annual exacerbation rate* | 0.296 (0.797) |
| Induced sputum | |
| Neutrophils (%) | 69.8 (42.4-88.3) |
| Eosinophils (%) | 3.8 (0-21.3) |
| Macrophages (%) | 10.4 (2.9 - 27.3) |
| Lymphocytes (%)† | 0 (0 - 2.3) |

| | |
|---|---|
| Columnar cells (%)‡ | 0.5 (0 - 2.3) |
| FVC (% pred.) | 86 (69-96) |
| $FEV_1$ (% pred.) | 82 (66.5-100.5) |
| $FEV_1$/FVC (%) | 77 (67 - 84) |

Median (interquartile range)

* Mean (standard deviation)

NS non-smoker, ES ex-smoker,

**Table 3**. Characterization of asthmatic patients (n = 89) with or without sputum IL-32

| | IL-32-positive patients | IL-32-negative patients | p-value |
|---|---|---|---|
| Subjects (n) | 25 | 64 | |
| Sex, % female | 80 | 77 | 0.727 |
| Age (yr) | 46.7 (31.0 - 57.3) | 53.6 (40.3 – 62.0) | 0.097 |
| BMI ($kg/m^2$) | 24.7 (20.7 - 26.8) | 24.5 (21.6 - 26.6) | 0.688 |
| Ex-smoker,% (n) | 12 (3/25) | 11 (7/64) | 0.887 |
| Age of asthma onset | 41.4 (25.6 – 54.2) | 48.9 (37.8 – 58.3) | 0.034 |
| Steroid treatments | 20% (5/25) | 9% (6/64) | 0.171 |
| Total follow-up periods | 5.0 (2.7 – 8.5) | 3.5 (0.7 - 8.5) | 0.738 |
| Asthma exacerbation rate * | 86.8 | 16.2 | **0.030** |
| PC20† | 4.4 (0.8 - 11.6) | 10.9 (1.5 - 25.0) | 0.064 |
| Total serum IgE | 171.0 (58.7 – 359.5) | 82.7 (26.6 – 262.8) | 0.122 |
| Blood eosinophils (/ul) | 209 (147–766) | 309 (120–636) | 0.784 |

| | | | |
|---|---|---|---|
| FVC (% pred.) | 69.0 (61.5 – 90.0) | 87.0 (75.3 – 98.0) | 0.011 |
| $FEV_1$ (% pred.) | 72.0 (54.0–89.0) | 89.0 (69.3 -107.8) | 0.010 |
| Sputum neutrophil% | 69.3 (30.7- 88.6) | 71.3 (46.0 - 88.3) | 0.391 |
| Sputum eosinophil% | 2.3 (0.0 – 21.8) | 4.1 (0.0 - 21.9) | 0.454 |
| IL-33 (ng/ml) | 0.39 (0.00 - 0.88) | 0.31 (0.09 - 0.58) | 0.812 |

Median (interquartile range)

* Per 100 person years

† Data from 22 IL-32 (+) patients and 52 IL-32 (−) patients

**Figure 1.** Induction of IL-32 mRNA in BEAS2B cells. BEAS2B cells were stimulated with Poly I:C (TLR 3 ligand), house dust allergen (Dp), cigarette smoking extract (CSE). Results are expressed as arithmetic mean plus SD; *P < 0.05; **P < 0.01, when compared with medium alone.

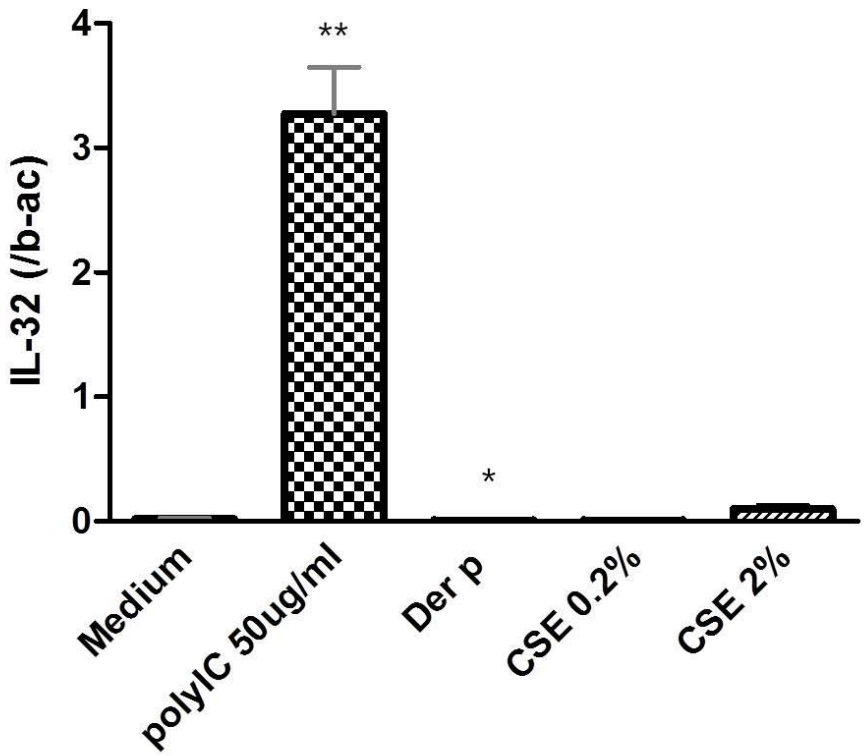
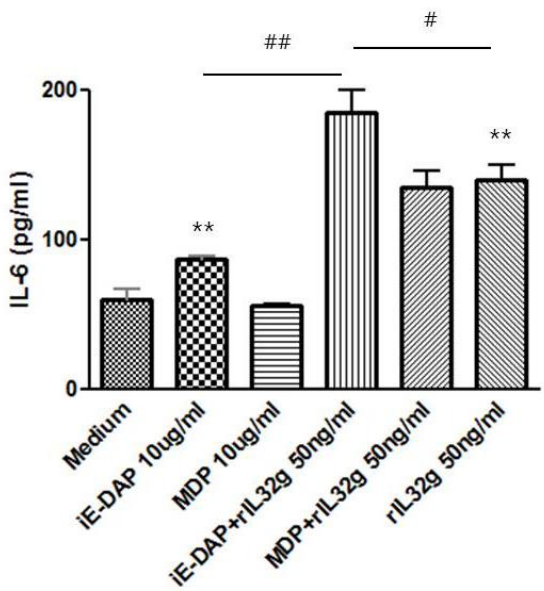
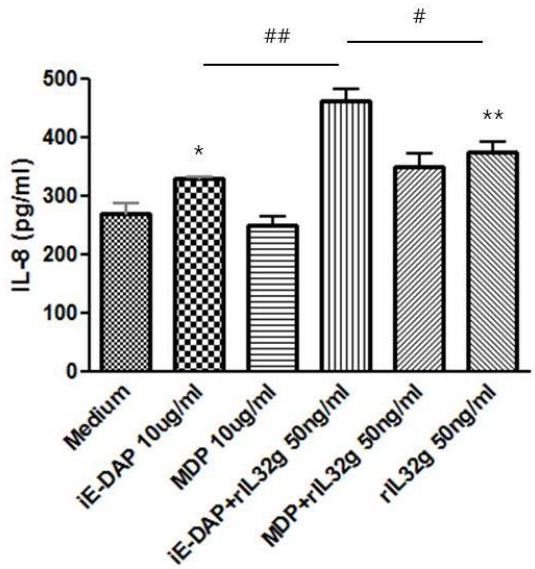


**Figure 2.** Stimulation with IL-32 (50 ng/ml) and NOD1, 2 ligands for induction of IL-6 (A), IL-8 (B), and IL-33 mRNA (C). BEAS2B cells were stimulated with IL-32 and NOD ligands (10 μg/ml). Results are expressed as arithmetic mean plus SD; *P < 0.05; **P < 0.01, when compared with medium alone; #P < 0.05; ##P < 0.01. iE-DAP/MDP+IL-32g, combined treatment of iE-DAP/MDP with IL-32γ.

(A)



(B)


##
#
500
400
300
200
100
0
IL-8 (pg/ml)
*
**
Medium
iE-DAP 10ug/ml
MDP 10ug/ml
iE-DAP+rIL32g 50ng/ml
MDP+rIL32g 50ng/ml
rIL32g 50ng/ml

(C)

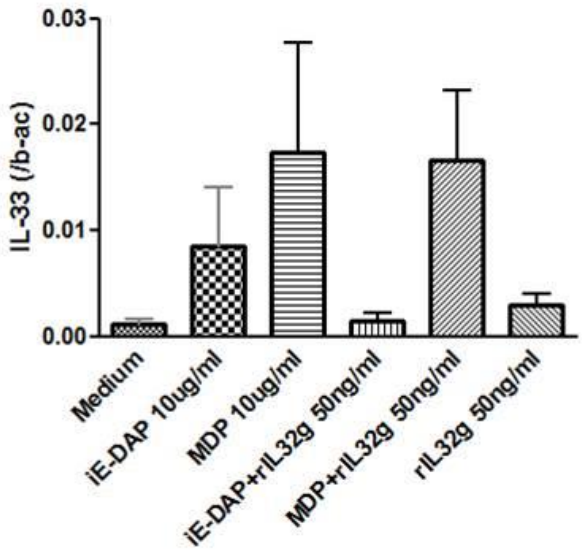
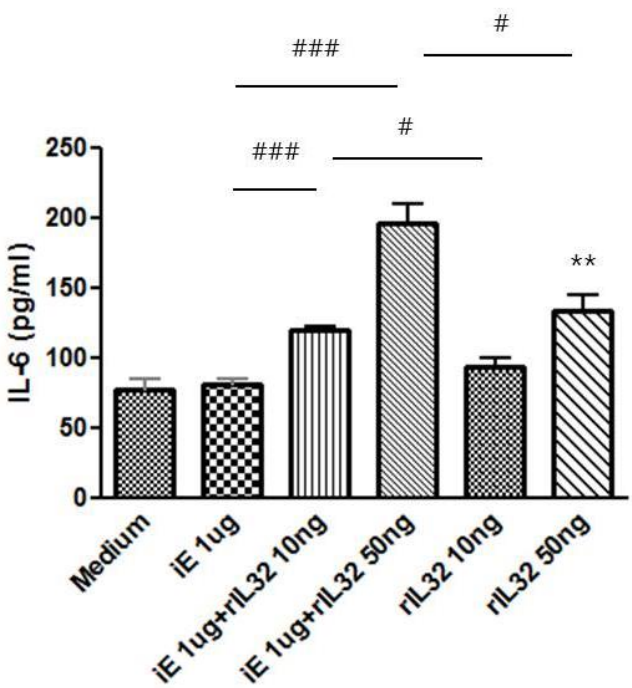
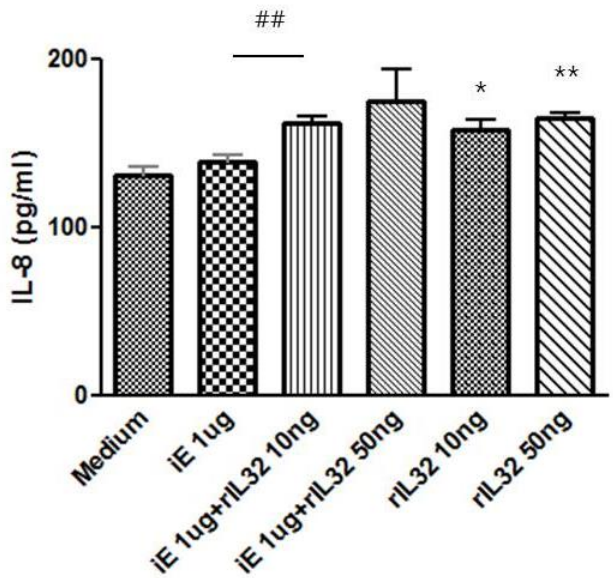

0.03
0.02
0.01
0.00
IL-33 (/b-ac)
Medium
iE-DAP 10ug/ml
MDP 10ug/ml
iE-DAP+rIL32g 50ng/ml
MDP+rIL32g 50ng/ml
rIL32g 50ng/ml

**Figure 3.** Stimulation with low dose NOD1 ligands (iE) and IL-32 for the induction of IL-6, IL-8, and IL-33. Results are expressed as arithmetic mean plus SD; *P < 0.05; **P < 0.01, when compared with medium alone; #P < 0.05; ##P < 0.01; ###P < 0.001. iE+IL-32, combined treatment of iE-DAP with IL-32γ.

(A)



(B)


##
*
**
200
100
0
IL-8 (pg/ml)
Medium
iE 1ug
iE 1ug+rIL32 10ng
iE 1ug+rIL32 50ng
rIL32 10ng
rIL32 50ng

(C)

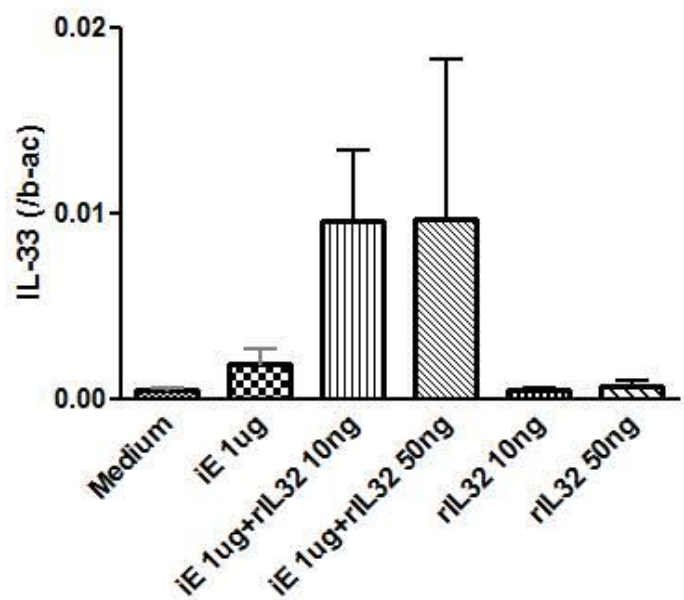
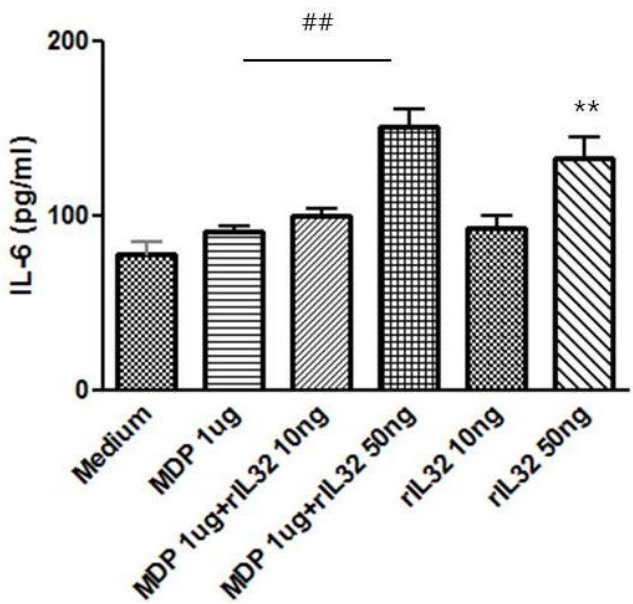
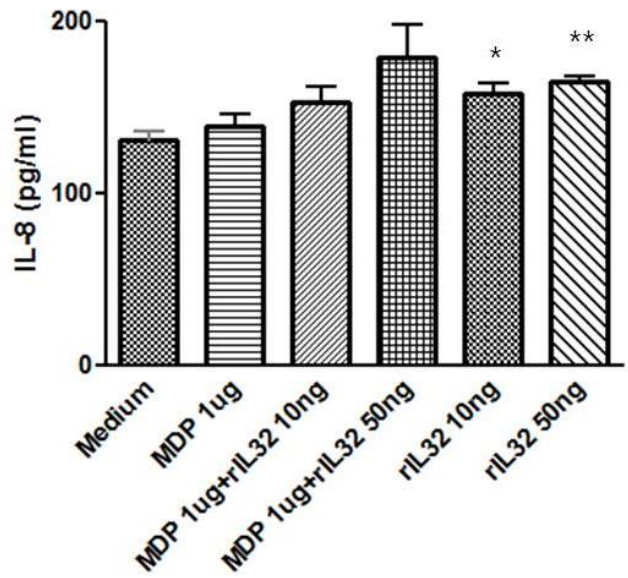

0.02
0.01
0.00
IL-33 (/b-ac)
Medium
iE 1ug
iE 1ug+rIL32 10ng
iE 1ug+rIL32 50ng
rIL32 10ng
rIL32 50ng

**Figure 4.** Stimulation with low dose NOD2 ligands (MDP) and IL-32 for the induction of IL-6, IL-8, and IL-33. Results are expressed as arithmetic mean plus SD; *P < 0.05; **P < 0.01, when compared with medium alone; ##P < 0.01. MDP +IL-32, combined treatment of MDP with IL-32γ.

(A)



(B)


200
100
0
IL-8 (pg/ml)
*
**
Medium
MDP 1ug
MDP 1ug+rIL32 10ng
MDP 1ug+rIL32 50ng
rIL32 10ng
rIL32 50ng

(C)

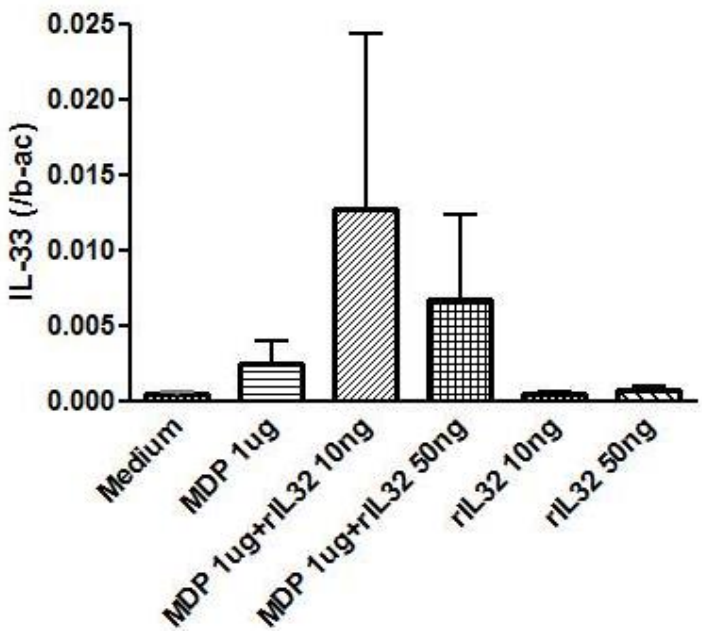

0.025
0.020
0.015
0.010
0.005
0.000
IL-33 (/b-ac)
Medium
MDP 1ug
MDP 1ug+rIL32 10ng
MDP 1ug+rIL32 50ng
rIL32 10ng
rIL32 50ng

**Figure 5.** Induction of IL-32 mRNA in BEAS2B cells. BEAS2B cells were stimulated with low dose NOD1, 2 ligands and IL-32. Results are expressed as arithmetic mean plus SD.

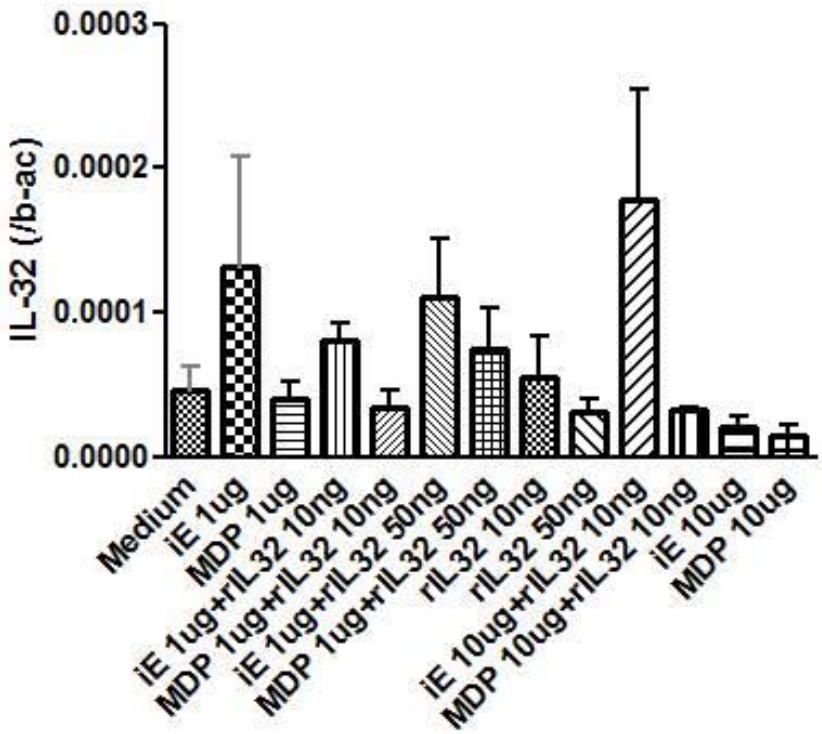
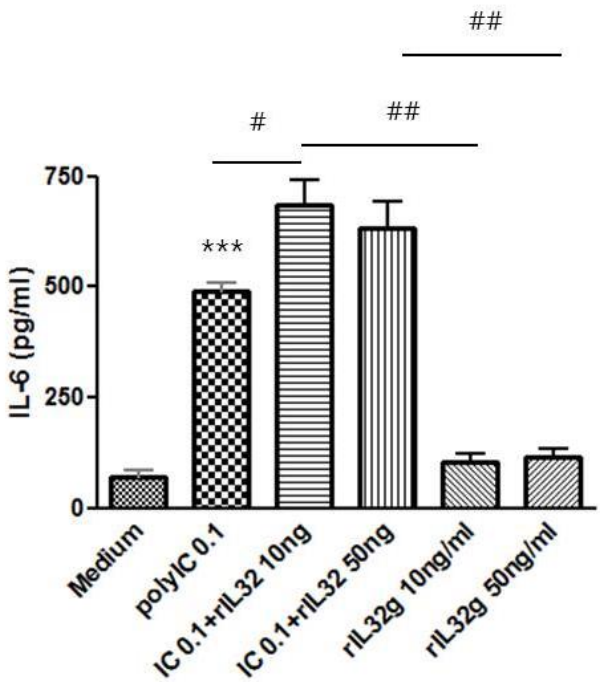
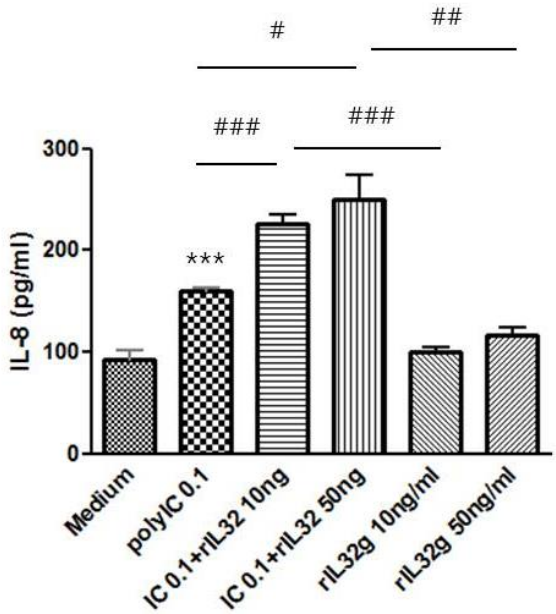


**Figure 6.** Stimulation with Poly I:C (TLR 3 ligand) and IL-32 for the induction of IL-6, IL-8, and IL-33. Results are expressed as arithmetic mean plus SD; * P < 0.05; **P < 0.01, when compared with medium alone; ##P < 0.01; ###P < 0.001. IC+IL-32, combined treatment of Poly I:C with IL-32γ.

(A)



(B)


#
##
###
###
300
200
100
0
IL-8 (pg/ml)
***
Medium
polyIC 0.1
IC 0.1+rIL32 10ng
IC 0.1+rIL32 50ng
rIL32g 10ng/ml
rIL32g 50ng/ml

(C)

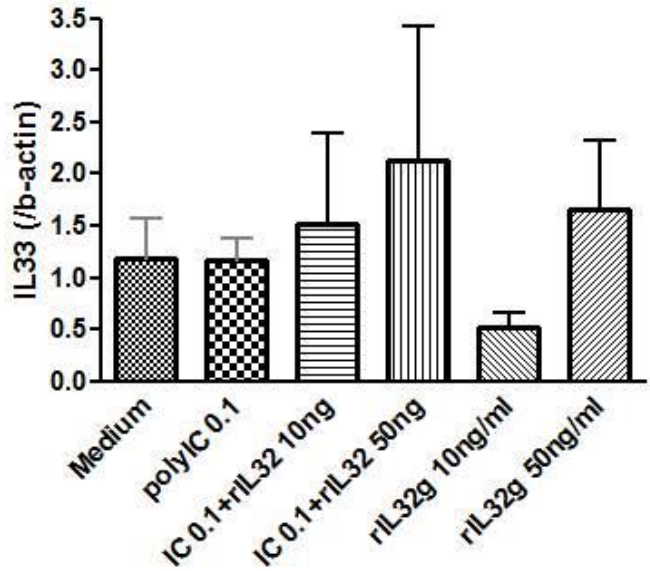
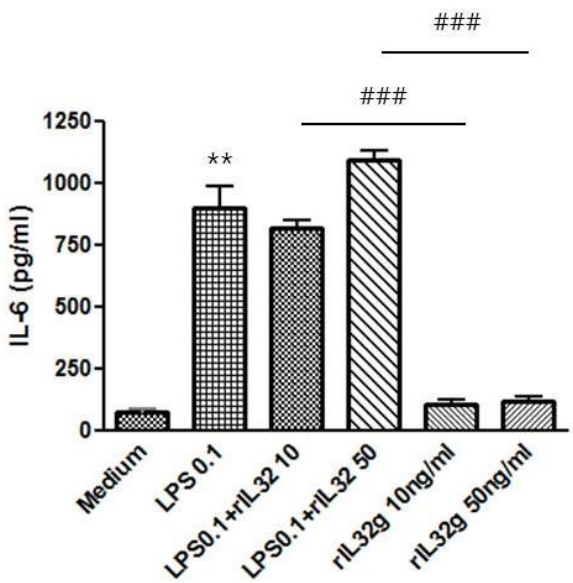
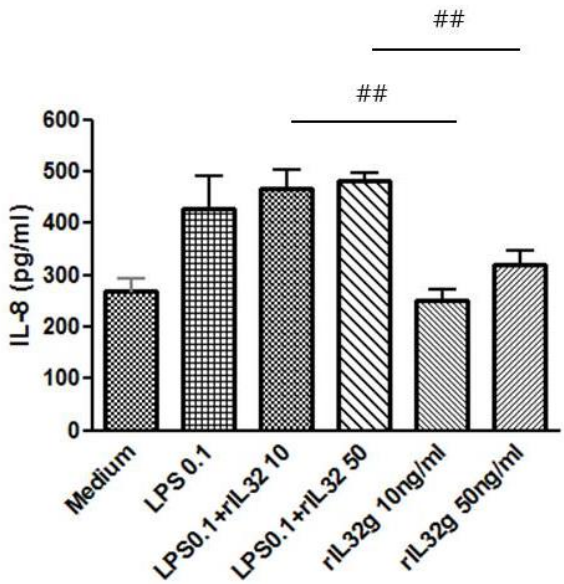

3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
IL33 (/b-actin)
Medium
polyIC 0.1
IC 0.1+rIL32 10ng
IC 0.1+rIL32 50ng
rIL32g 10ng/ml
rIL32g 50ng/ml

**Figure 7.** Stimulation with LPS (TLR4 ligand) and IL-32 for the induction of IL-6, IL-8, and IL-33. Results are expressed as arithmetic mean plus SD; *P < 0.05; **P < 0.01, when compared with medium alone; ##P < 0.01. LPS+IL-32, combined treatment of LPS with IL-32γ.

(A)



(B)


##
##
600
500
400
300
200
100
0
IL-8 (pg/ml)
Medium
LPS 0.1
LPS0.1+rIL32 10
LPS0.1+rIL32 50
rIL32g 10ng/ml
rIL32g 50ng/ml

(C)

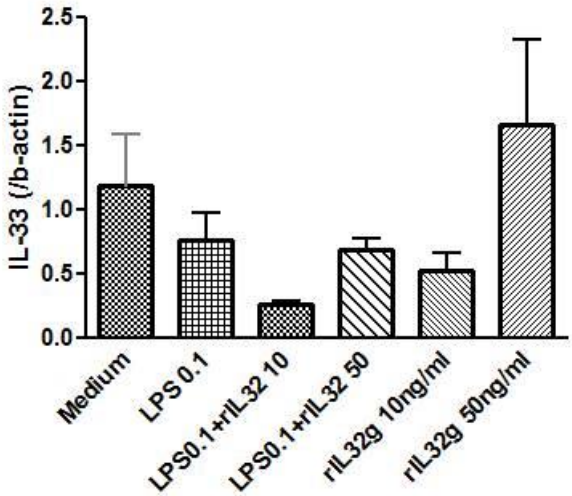
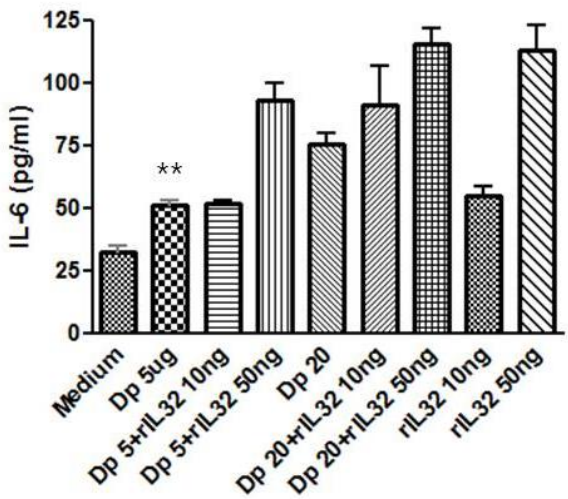
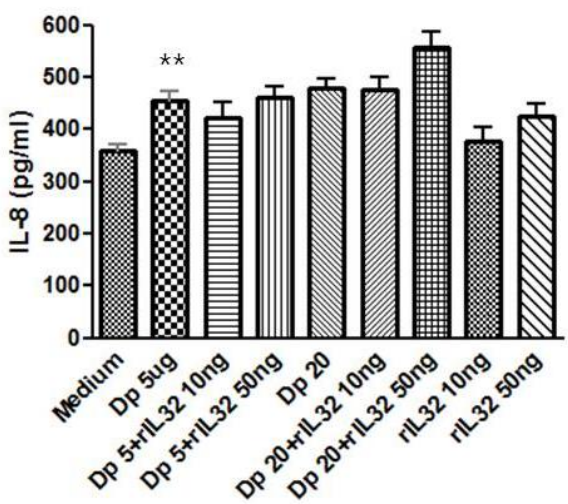

2.5
2.0
1.5
1.0
0.5
0.0
IL-33 (/b-actin)
Medium
LPS 0.1
LPS0.1+rIL32 10
LPS0.1+rIL32 50
rIL32g 10ng/ml
rIL32g 50ng/ml

**Figure 8.** Stimulation with house dust allergen (Dp) and IL-32 for the induction of IL-6, IL-8, and IL-33. Results are expressed as arithmetic mean plus SD; *P < 0.05; **P < 0.01, when compared with medium alone; ##P < 0.01. DP+IL-32, combined treatment of DP with IL-32γ.

(A)




(B)



(C)

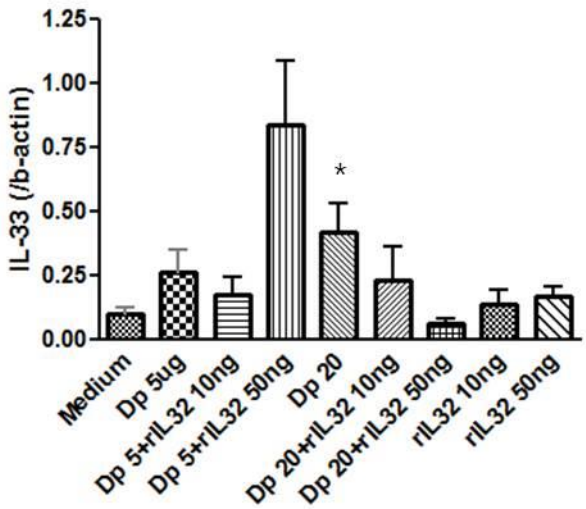
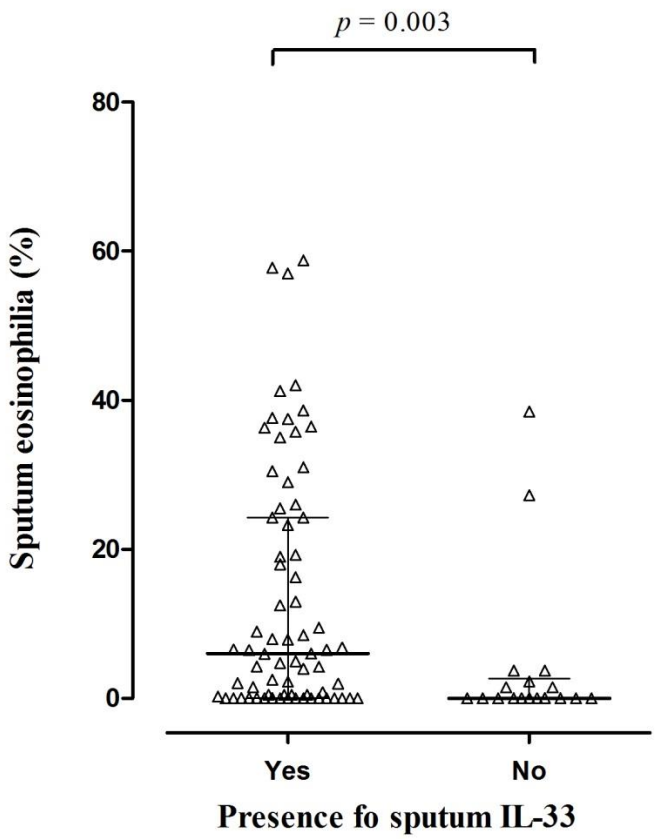

IL-33 (/b-actin)
1.25
1.00
0.75
0.50
0.25
0.00
*
Medium
Dp 5ug
Dp 5+rIL32 10ng
Dp 5+rIL32 50ng
Dp 20
Dp 20+rIL32 10ng
Dp 20+rIL32 50ng
rIL32 10ng
rIL32 50ng

**Figure 9.** Plasma levels of IL-32 and IL-33 between normal control and patients with asthma. Date is presented as median with interquartile range. Date is presented as median with interquartile range.

(A)



(B)

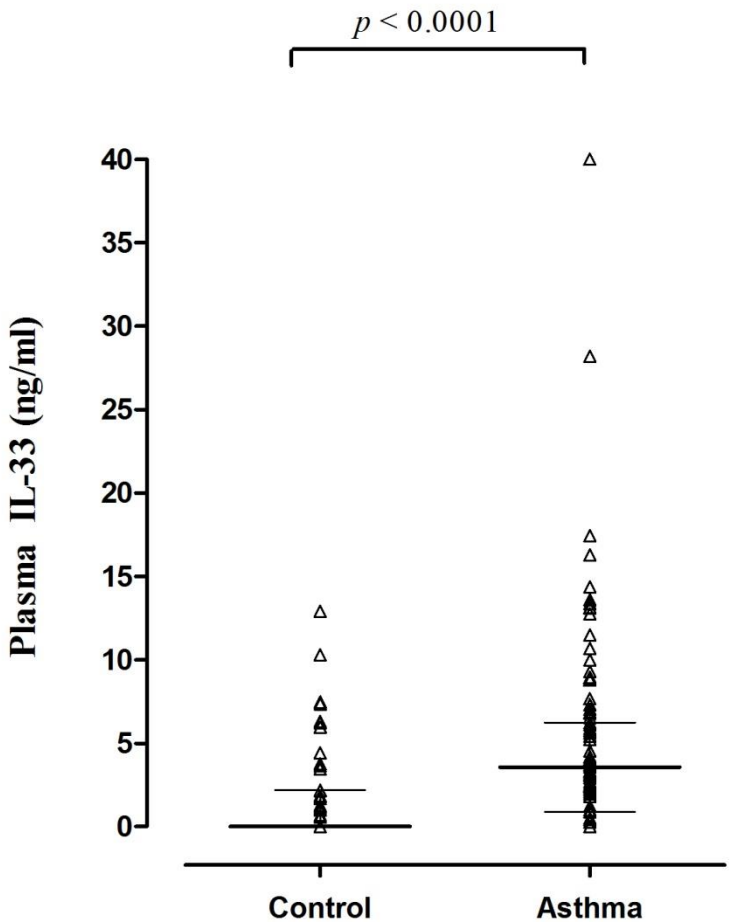
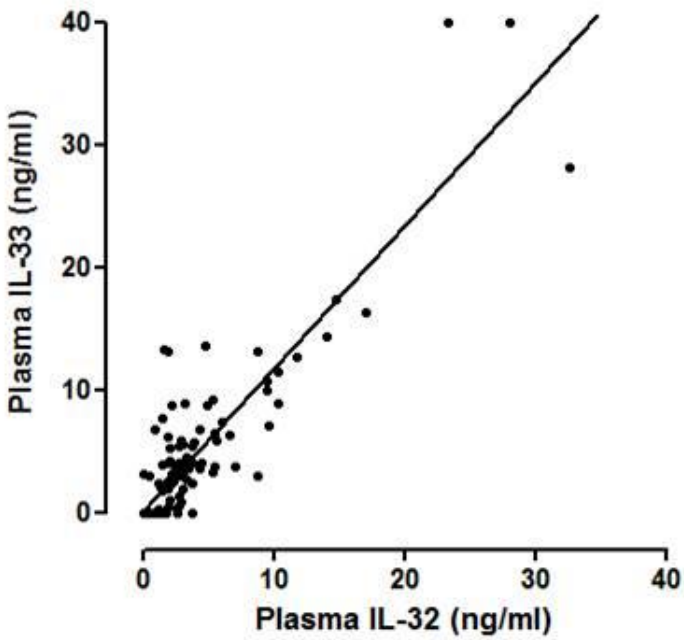


**Figure 10.** Correlation between plasma levels of IL-32 and IL-33 in (A) asthmatic patients (r = 0.720, $p < 0.0001$), and (B) normal control (r = 0.784, $p < 0.0001$).

(A) Asthma



(B) Normal control

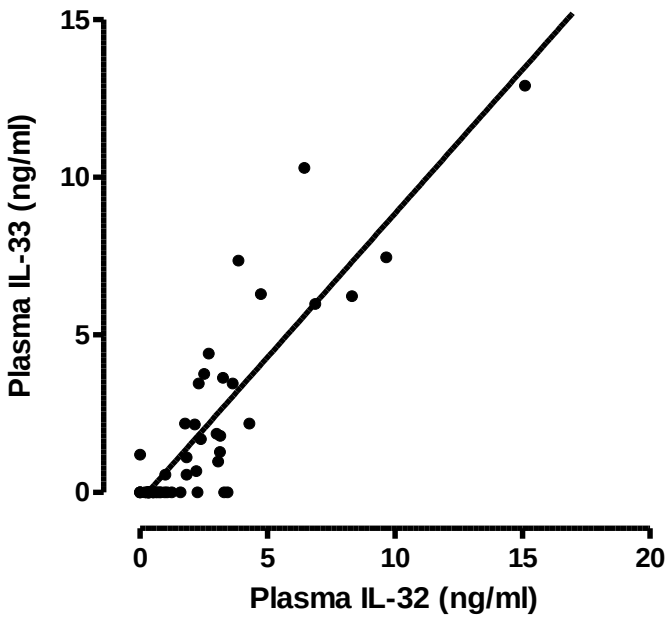
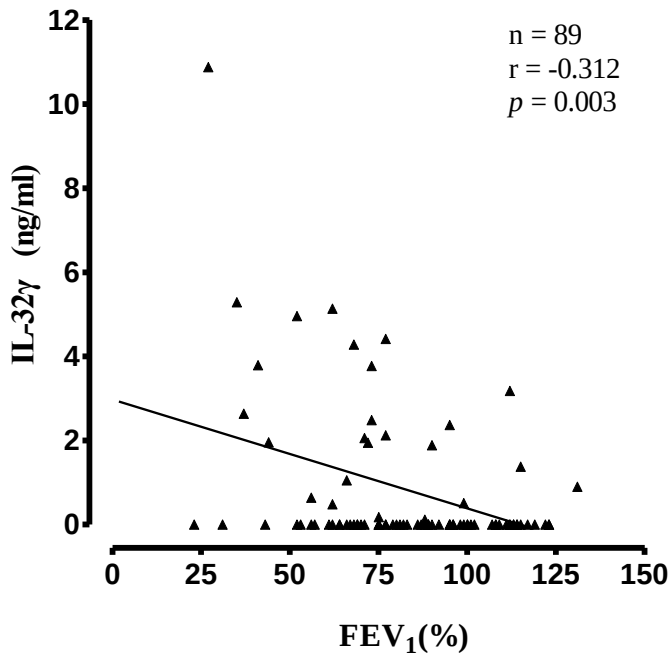


**Figure 11.** Correlation between the level of sputum IL-32 and $FEV_1$ (% pred.) (A), and between the level of sputum IL-32and asthma exacerbations (B) in patients with stable asthma (n = 89).

(A)



(B)

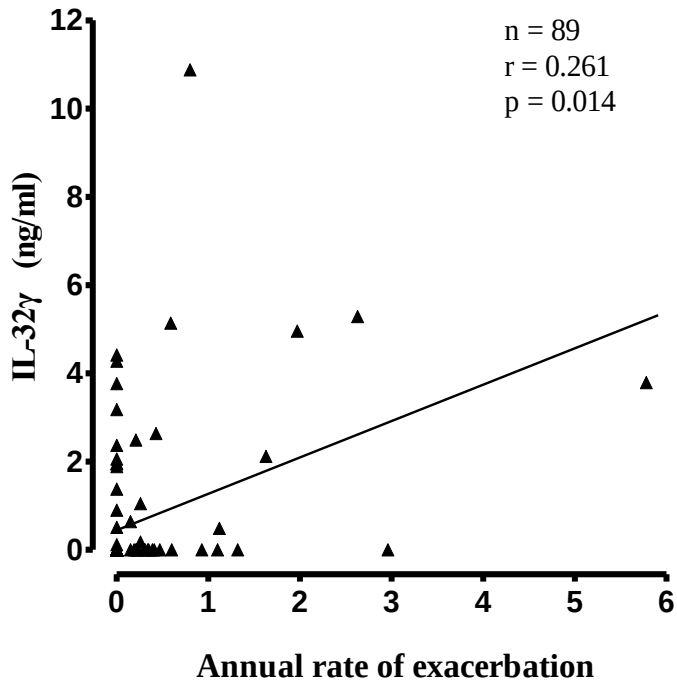

n = 89
r = 0.261
p = 0.014
IL-32γ (ng/ml)
12
10
8
6
4
2
0
0
1
2
3
4
5
6
Annual rate of exacerbation

**Figure 12.** Comparison of annual disease exacerbation rates between asthma patients with detectable and non-detectable sputum IL-32. Date is presented as median with interquartile range.

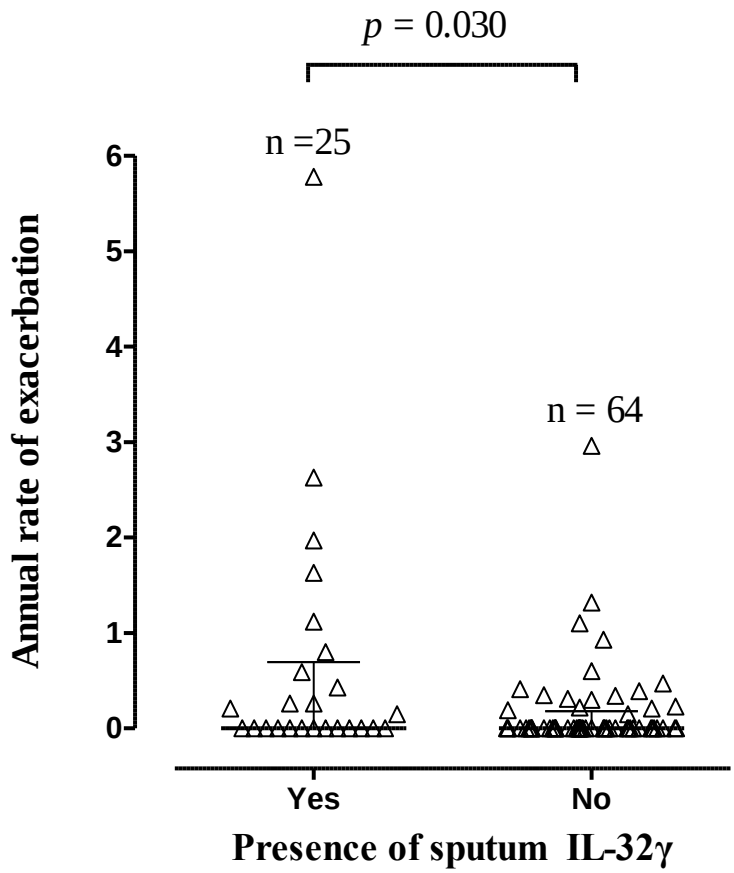
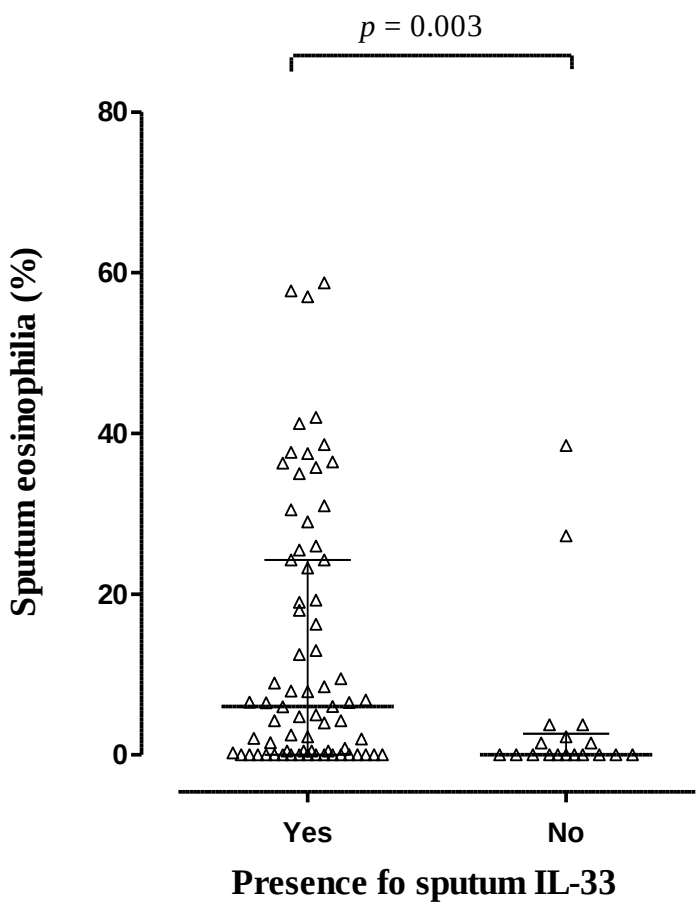
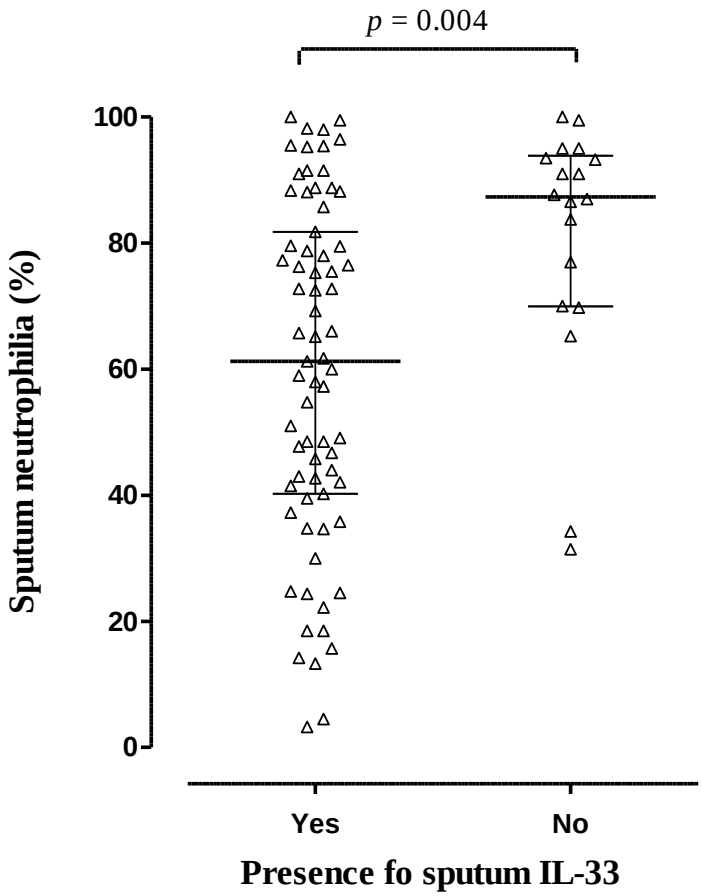


**Figure 13.** Comparison of inflammatory profiles of induced sputum according to the presence of IL-33 in induced sputum of patients with asthma. (A) sputum eosinophilia, (B) sputum neutrophilia. Date is presented as median with interquartile range.

(A)



(B)


p = 0.004
Sputum neutrophilia (%)
100
80
60
40
20
0
Yes
No
Presence fo sputum IL-33

# 국문초록

서론: 선천 면역과 감염은 천식 발병 및 악화에 중요한 것으로 알려져 있다. IL-32는 다양한 감염성 자극에 대한 선천 면역 반응과 관련된 염증성 싸이토카인으로 류마티스관절염, 크론병, 만성폐쇄성폐질환 등과 같은 다양한 만성 염증성 질환에 연관된 것으로 보고되었다. 이 연구는 천식에서 IL-32의 역할을 평가하기 위해 수행되었다.

방법: 기관지에서 IL-32γ의 역할을 확인하기 위하여 기관지상피세포 세포주(BEAS2B)를 천식의 악화인자로 알려진 NOD 리간드, dsRNA, lipopolysaccharide (LPS), 집먼지진드기 항원(Der p) 등으로 자극하였다. 또한 IL-32γ를 천식 환자(n = 103)와 건강한 대조군(n = 51)의 혈장에서 측정하였고, 천식 환자의 유도객담상층액(n = 89)에서 측정하였다. 측정된 IL-32γ 발현 정도와 기도폐쇄 ($FEV_1$), 유도객담의 염증패턴 (호중구 및 호산구 염증), 천식의 악화빈도 등 천식의 임상 특성과 비교 평가하였다.

결과: 실험 연구에서, IL-32γ는 NOD 1 리간드 및 dsRNA 자극에 의해 BEAS2B 로부터 IL-6가 분비되는 반응을 상승시키는 효과를 보였다. IL-32γ는 95% (98/103) 천식 환자의 혈장에서 발견되었고 천식 환자의 혈장 IL-32γ의 수치는 건강한 대조군의 혈장 IL-32γ 수치보다 높았다. 유도객담분석에서 IL-32γ는 천식 환자 89 명 중 25 명(28.1%)의 유도객담상층액에서 발견되었으며, 이러한 환자에서 천식

악화의 빈도는 유도객담에서 IL-32γ가 발견되지 않은 환자(n = 64)보다 더 높았다 ($p$ = 0.03).

결론: 이 연구는 기도의 IL-32γ가 감염성 자극에 대한 염증 반응을 증가시킬 수 있으며, 객담에 IL-32γ가 분비되는 일부의 천식환자에서 IL-32γ가 천식 악화에 기여할 수 있음을 시사한다.

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**주요어:** : IL-32, 천식, 악화

**학 번:** 2010-30562